AMENDMENT UNDER 37 C.F.R. §1.312 Attorney Docket No.: Q85108

Application No.: 10/517,422

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the

application:

LISTING OF CLAIMS:

1. (currently amended): A method for classifying and counting leukocytes, which

comprises:

(1) a step of staining cells in a hematological sample by treatment with a hemolytic

agent, with a fluorescent dye capable of differentiating between fluorescence intensity, mature

leukocytes, leukocytes with abnormal DNA amount and immature leukocytes a step of staining

cells in a hematological sample by treatment with a hemolytic agent, with a fluorescent dye

capable of differentiating in fluorescence intensity between mature leukocytes, leukocytes with

abnormal DNA amount and immature leukocytes;

(2) a step of introducing the sample containing the stained cells into a flow cytometer to

measure first scattered light, second scattered light different from the first scattered light and

fluorescence of the cells;

(3) a step of obtaining scattered light peak intensities and scattered light widths of the

cells based on the measured first scattered light, obtaining scattered light intensities of the cells

based on the measured second scattered light, and obtaining fluorescence intensities of the cells

based on the measured fluorescence light;

(4) a step of classifying the cells into a first group and a second group based on the

scattered light peak intensities and the scattered light widths, the first group including leukocytes

and the second group including coincidence cells and platelet clumps;

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(5) a step of classifying the leukocytes included in the first group into mature leukocytes, leukocytes with abnormal DNA amount and immature leukocytes based on the scattered light intensities and the fluorescence intensities of the leukocytes included in the first group; and

- (6) a step of counting the classified mature leukocytes, the classified leukocytes with abnormal DNA amount and the classified immature leukocytes.
- 2. (original): The method according to claim 1, which further comprises a step of calculating a ratio of mature leukocytes or immature leukocytes relative to leukocytes with abnormal DNA amount from a number of leukocytes with abnormal DNA amount and a number of mature leukocytes or immature leukocytes.
- 3. (previously presented): The method according to claim 1, which further comprises a step of calculating a ratio of immature leukocytes relative to mature leukocytes from a number of mature leukocytes and a number of immature leukocytes.

4 - 5. (canceled).

6. (previously presented): The method according to claim 1, wherein the fluorescent dye is selected from the group consisting of a compound represented by the formula (I):

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(wherein R¹¹ is a hydrogen atom or a lower alkyl group; R²¹ and R³¹ each is a hydrogen atom, a lower alkyl group or a lower alkoxy group; R⁴¹ is a hydrogen atom, an acyl group or a lower alkyl group; R⁵¹ is a hydrogen atom or a lower alkyl group which may be substituted; Z is sulfur atom, oxygen atom, or carbon atom which is substituted by a lower alkyl group; n¹ is 1 or 2; and X¹⁻ is an anion), ethidium bromide, propidium iodide, ethidium-acridine heterodimer, ethidium azide, ethidium homodimer-1, ethidium homodimer-2, ethidium monoazide, TOTO-1, TOTO-3, and TO-PRO-3.

- 7. (previously presented): The method according to claim 1, wherein the hemolytic agent comprises the following components:
 - (1) a polyoxyethylene nonionic surfactant;
- (2) a solubilizing agent which damages cell membrane of blood corpuscles and reduce their size;
 - (3) an amino acid; and
- (4) a buffer having a pH range adjusted to 5.0 9.0 and osmotic pressure adjusted to 150 600 mOsm/kg.
- 8. (previously presented): The method according to claim 7, wherein the polyoxyethylene nonionic surfactant comprises a compound represented by the following formula (II):

$$R^{III}$$
- R^{ZII} -(CH₂CH₂O)n_{II}-H (II)

(wherein R^{III} represents a C₉₋₂₅ alkyl, alkenyl or alkynyl group; R^{2II} represents -O-,

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or -COO-; and n_{II} is 10-40).

9. (previously presented): The method according to claim 7, wherein the solubilizing agent is a compound selected from the group consisting of

a sarcosine derivative of the formula (III):

$$\begin{array}{c|c} & \text{O} & \text{CH}_3 \\ \parallel & \mid & | & \text{(III)} \\ \text{R}^{1 \parallel \text{III}} & \text{--} \text{COOH} \end{array}$$

(wherein $R^{1 I I I}$ is a $C_{10\text{-}22}$ alkyl group; and $n^{I I I}$ is 1-5)

or salts thereof;

a cholic acid derivative of the formula (IV):

$$\begin{array}{c} & & & \\ & &$$

(wherein R^{1IV} is a hydrogen atom or a hydroxy group);

and

a methylglucanamide of the formula (V):

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$$H_3C$$
— $(CH_2)_{nV}$ — CH_2 N OH OH OH (V)

(wherein n^V is 5-7).

- 10. (previously presented): The method according to claim 1, wherein scattered light to be measured is selected from forward low angle scattered light, forward high angle scattered light and side scattered light.
 - 11. (canceled).
- 12. (previously presented): The method according to claim 1, wherein the classifying step (5) is performed so as to classify the classified mature leukocytes into at least three groups based on the scattered light intensities.
- 13. (previously presented): The method according to claim 1, wherein the classifying step (5) is performed so as to classify the classified immature leukocytes into at least two groups based on the scattered light intensities.